The susceptibility of organisms associated with *bacterial vaginosis* to spermicidal compounds, in vitro

Brian M Jones, Lorna M Willcox

Abstract

***Objectives*—Bacterial vaginosis (BV) is a prevalent vaginal infection that is now regarded as a risk factor in more serious pelvic and obstetric complications. Spermicides are known to have antimicrobial activity against other sexually transmitted diseases and the aim of this study was to test whether the causative organisms of BV were also susceptible to spermicides, in vitro.**

***Design*—Minimal Inhibitory Concentrations of five spermicidal compounds were determined for the organisms associated with BV, in an agar dilution technique.**

***Location*—The Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, UK.**

***Spermicides and Organisms*—Nonoxynol-9, Nonoxynol-11, Docusate sodium, Benzalkonium chloride and Menfegol were tested against 20 strains each of *Gardnerella vaginalis*, *Bacteroides* and *Mobiluncus* organisms, isolated from patients with BV who attended the Department of Genitourinary Medicine, the Royal Hallamshire Hospital, Sheffield.**

***Main outcome measures*—The susceptibility of BV-associated organisms to spermicidal compounds, in vitro.**

***Results*—*G vaginalis*, *Mobiluncus* spp, *Bacteroides bivius* and *Bacteroides disiens* were all susceptible to the five spermicides tested, with MICs ranging between ≤19 and 5000 mg/l (0·0019%–0·5%).**

***Conclusion*—The concentrations of spermicides incorporated in contraceptive preparations are usually between 3% and 8%, which are far in excess of the MICs found for BV organisms. Their usage could exert a significant antimicrobial effect and be a useful prophylactic in preventing the infection.**

Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, UK
Brian M Jones

The London International Group, PLC, Unit 203, Cambridge Science Park, Cambridge, CB4 4EZ, UK
Lorna M Willcox

Introduction

The active ingredients of spermicidal preparations are known to have antimicrobial activity in vitro against many of the causative agents of sexually transmitted diseases, including *Treponema pallidum*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, Herpesvirus, types I and II, the Human Immunodeficiency Virus, *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Haemophilus ducreyi*.[1,2] Clinical studies have confirmed that spermicides, particularly when combined with the use of condoms or diaphragms, can also provide effective prophylaxis against these infections in vivo.[3]

Bacterial vaginosis (BV) has now become the commonest vaginal infection known[4] and, so far, the susceptibility of its causative organisms to spermicide compounds has not been evaluated. This study determined the Minimal Inhibitory Concentration of five spermicidal compounds for *Gardnerella vaginalis*, *Mobiluncus* and *Bacteroides* organisms, in an agar-dilution technique.

Materials and methods

Spermicide compounds

The spermicide compounds tested were Nonoxynol-9 (N-9), Nonoxynol-11 (N-11), Benzalkonium chloride (BZK), Sodium dodecyl sulpho-succinate (docusate sodium) and α((p-methyl)W-hydroxypoly (Oxyethylene)-(Menfegol). All were supplied by the London International Group, PLC, Cambridge, CB4 4G7, UK.

Culture media

The medium used for culture and maintenance of the test strains was Columbia agar base, enriched with defibrinated horse blood, 7·5% and, for MIC determinations, DST agar, enriched with lysed, defibrinated horse blood, 5% (Oxoid Ltd, Basingstoke, RG24 0PW, UK).

Organisms

Twenty strains each of *G vaginalis*, *Mobiluncus* and *Bacteroides* organisms were used in the study. All had been isolated from vaginal specimens, taken from patients with BV who had attended the Department of Genitourinary Medicine, the Royal Hallamshire

Hospital, Sheffield. They were stored in liquid nitrogen vapour phase, at −130°C, prior to use.

MIC determinations

An agar-dilution method was used. The pure spermicide compounds were weighed and dissolved in sterile distilled water to make 10% stock solutions. A range of doubling dilutions were made, in 5 ml volumes, to provide solutions from 10% to 0·019%. Five ml of these dilutions, together with 2·5 ml of lysed horse blood were added to 42·5 ml molten and cooled DST agar and plates poured at concentrations ranging from 1% (10 000 mg/l) to 0·0019% (19 mg/l). To overcome solubility problems, plates containing 5% and 2·5% were prepared by adding the spermicides directly to molten DST agar.

Colonies of the organisms to be tested were suspended in sterile, physiological saline and diluted to circa 10^9 cfu/ml, by comparison with McFarland standard opacity tubes (API-bio Merieux Ltd, Basingstoke, RG22 6HY, UK), previously calibrated by surface viable counts. A multipoint inoculator (Denley Instruments Ltd, Billinghurst, RH14 9EY, UK) was used to deliver 0·001 ml (circa 10^6 cfu) to the surface of the spermicide-containing plates. *G vaginalis* cultures were incubated at 37°C, in an atmosphere of 10% CO_2 in air for two days: *Bacteroides* and *Mobiluncus* spp were incubated at 37°C, in an anaerobic cabinet (Don Whitley Scientific, Shipley, BD17 7SE, UK) in an atmosphere of 80% N_2, 10% H_2 and 10% CO_2, for two or four days, respectively. The MICs were recorded as the lowest concentration that prevented visible growth.

Results

MIC determination (table) showed *G vaginalis* and *Mobiluncus* spp to be highly susceptible to all five spermicides, with benzalkonium chloride having the strongest activity. The susceptibility of *Bacteroides* organisms, however, was variable. All strains were highly susceptible to benzalkonium chloride and Docusate sodium but only nine of the 19 strains were susceptible to Nonoxynol-9, Nonoxynol-11 and Menfegol at concentrations of 1% or less. These susceptible strains, belonged to the *B bivius* and *B disiens* groups, the types most commonly found in BV, whereas the relatively resistant strains were of the *B fragilis* and *B oralis* groups, which are not usually found in BV.

Discussion

Vaginal infections affect large numbers of women with a variety of symptoms and sequelae that vary widely in severity. BV is now the most prevalent and rather than merely being the cause of an unpleasant discharge, has become a risk factor in more serious pelvic and obstetric infections.[4]

This study has shown the organisms associated with BV to be susceptible to spermicide compounds, in vitro and the concentrations incorporated into contraceptive preparations, usually between 3% and 8%, are far in excess of the MICs found for *G vaginalis*, *Mobiluncus* and *Bacteroides* organisms. These in vitro findings suggest that the use of spermicides could exert a significant antimicrobial effect against BV, in vivo and, together with barrier

Table Minimum inhibitory concentrations of spermicide compounds against the organisms associated with Bacterial vaginosis, in vitro (mg/l)

Spermicide	*G vaginalis (n = 20)*	*Bacteroides spp (n = 19)*	*Mobiluncus spp (n = 20)*
Nonoxynol-9			
MIC range	39–156	39–50 000	39–156
MIC^{50}	78	25 000	78
MIC^{90}	156	50 000	156
Nonoxynol-11			
MIC range	39–156	39–>50 000	78–312
MIC^{50}	156	25 000	78
MIC^{90}	156	>50 000	312
Benzalkonium chloride			
MIC range	⩽19	⩽19–39	⩽19
MIC^{50}	⩽19	⩽19	⩽19
MIC^{90}	⩽19	39	⩽19
Docusate sodium			
MIC range	78–2500	78–5000	312–1250
MIC^{50}	2500	2500	625
MIC^{90}	2500	5000	1250
*Menfegol**			
Mic range	39–312	⩽19–⩾10 000	78–156
MIC^{50}	156	⩾10 000	78
MIC^{90}	156	⩾10 000	156

*1% was the highest concentration tested.

contraception, which has been shown previously to reduce the incidence of BV,[5] could be effective prophylaxis in preventing the disease.

We are grateful to Mr I Geary and Mrs M E Lee for skilled technical help and to Mrs H Storer for her valuable secretarial assistance in preparing this manuscript. The financial support of the London International Group is gratefully acknowledged.

Address for correspondence: Dr B M Jones, Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, UK.

1 Judson FN, Ehret JM, Bodin GF, Levin MJ, Reitmeijer AM. *In vitro* evaluations of condoms with and without Nonoxynol-9 as physical and chemical barriers against *Chlamydia trachomatis*, Herpes simplex virus 2 and Human Immunodeficiency virus. *Sex Transm Dis* 1989;16:51–6.
2 Jones BM, Geary I, Lee ME, Duerden BI. Susceptibility of *Haemophilus ducreyi* to spermicidal compounds, in vitro. *Genitourin Med* 1991;67:268–9.
3 Feldblum PJ, Fortney JA. Condoms, spermicides and the transmission of Human Immunodeficiency Virus: A review of the literature. *Am J Public Health* 1988;78:52–4.
4 Swedberg JA. Bacterial vaginosis: etiology, association with preterm labour, diagnosis and management. *Compre Ther* 1989;15:47–53.
5 Bramley HM, Dixon RA, Jones BM. *Haemophilus vaginalis* (*Corynebacterium vaginale, Gardnerella vaginalis*) in a family planning population. *Br J Veneral Dis* 1981;57:62–6.

Accepted for publication 9 September 1991